

## UNCOUPLING AGENTS

This invention relates to uncoupling agents for use in the control of bacterial biomass in aqueous systems, to the use of such agents and to a  
5 method of using such agents.

Mechanistically, the generation of biomass in wastewater treatment arises from the consumption of nutrients in the wastewater. By a process of respiration, the nutrients are oxidised and this releases energy, which can  
10 be used by the microorganisms in cell division. If this energy could be "wasted", this would result in a reduced generation of biomass by inhibition of energy production

Bacterial biomass produced during the treatment of wastewater is costly  
15 to dispose of and so a reduction in biomass leads to reduced disposal costs.

Details of the biochemistry and mechanisms involved in cell respiration are given in, for example, "Biochemistry", 3<sup>rd</sup> edition, author: Lubert  
20 Stryer, publisher: W.H. Freeman & Company, New York 1988. Also in "General Microbiology", 3<sup>rd</sup> edition, authors: Roger Y. Stanier, Michael Doudoroff and Edward A. Adelberg, publisher: Macmillan, 1971. For the purposes of this patent application, the important point to note is that  
25 consumption of nutrients causes a proton flux which, in another part of the cell, is used to create adenosine triphosphate (ATP), via oxidative phosphorylation. ATP provides the energy for cell processes including cell division.

Hitherto, certain organic chemical compounds have been identified in the  
30 laboratory that will act as "uncoupling agents" to disrupt the proton flux. This results in a reduction in bacterial cell division by uncoupling the

proton flux thus reducing the energy available to the bacteria. The wasted energy appears as heat. The use of uncoupling agents can often cause increased nutrient consumption (which is desirable) due to the loss of respiratory control. However, the aforementioned uncoupling agents are usually phenolic compounds, e.g. 2,4-dinitrophenol and para-nitrophenol; halogenated products, e.g. carbonylcyanide-p-trifluoromethoxyphenylhydrazine and 2,4,5-trichlorophenol or nitro compounds. All are unsuitable for use in aqueous systems due to their high environmental toxicity.

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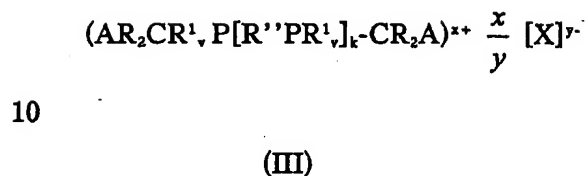
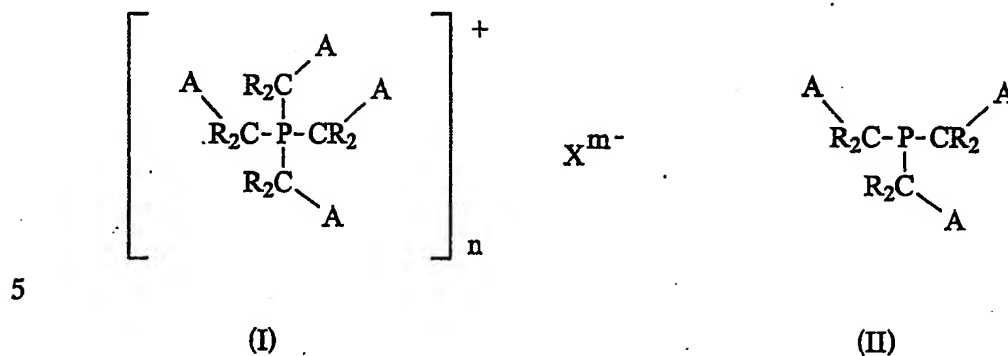
Accordingly the present invention provides the use of a water-soluble biocide as an uncoupling agent at an effective amount to control bacterial biomass in an aqueous system.

15 The effective amount of the water-soluble biocide added to the aqueous system is up to 5000mg/l, for example up to 3000mg/l, such as up to 1000mg/l. Preferably, the effective amount of the water-soluble biocide added to the aqueous system is from 0.005 mg/l to 500 mg/l, for example from 0.01mg/l to 300mg/l, such as from 0.05mg/l to 100mg/l. More  
20 preferably, the effective amount of water-soluble biocide is from 0.1 to 10 mg/l, for example from 0.5mg/l to 7.5mg/l, such as from 1 to 5mg/l.

Alternatively, the effective amount of said water-soluble biocide may be from 0.1 to 10,000 milligrams per gram of sludge solids in the aqueous  
25 system. Preferably, from 0.5 to 1000mg/g, for example, from 1 to 500mg/g, such as from 5 to 100mg/g.

In a preferred embodiment the current invention provides the use of a water-soluble biocide as an uncoupling agent, the water-soluble biocide  
30 comprising an alkyl-substituted phosphonium compound of formula (I), an

alkyl-substituted phosphine of formula (II) and a condensate of formula (III):



wherein:

X is an anion;

15 n is the valency of X represented by m;

each A can be the same or different and is selected from OH, OR, SO<sub>3</sub>R, PO<sub>3</sub>R<sub>2</sub>, COOH, COOR, SO<sub>3</sub>H, PO<sub>3</sub>H<sub>2</sub>, CH<sub>2</sub>COOH, substituted alkyl, aryl and substituted amino groups;

20 each R, and each R', when present, in each A group, is independently selected from hydrogen, a C<sub>1</sub> to C<sub>20</sub> alkyl, aryl, substituted alkyl or aryl, carboxy or carboxy ester; wherein each CR<sub>2</sub> group may be the same or different;

R'' is a divalent hydrocarbon radical having from 2-20 carbon atoms and is optionally substituted with one or more substituents selected from the group consisting of halogen, hydroxy, carboxy, amino, alkylamino, or  $\text{PR}^1\text{-CH}_2\text{OH}$  groups or interrupted by one or more ether or carbonyl linkages;

each  $\text{R}^1$  is independently a monovalent hydrocarbon radical having from 1 to 25 carbon atoms and optionally substituted with one or more substituents selected from the group consisting of halogen, hydroxy, carboxy, amino, alkylamino, or  $\text{PR}^1\text{-CH}_2\text{OH}$  groups or interrupted by one or more ether or carbonyl linkages; and

in formula (III) each  $v$  is 1 or 2,  $k$  is from 0 to 10 (e.g. from 1 to 10),  $x$  is the number of groups in the molecule having  $v=2$  and  $X$  is a compatible anion of valency  $y$  such that the compound is water-soluble.

$X$  is preferably selected from the group consisting of chloride, sulphate, phosphate, acetate and bromide.

The alkyl-substituted phosphonium compound is preferably tetrakis (hydroxymethyl) phosphonium sulphate. Alternatively, the alkyl-substituted phosphonium compound may be selected from the group consisting of tetrakis (hydroxymethyl) phosphonium chloride, tetrakis (hydroxymethyl) phosphonium bromide and tetrakis (hydroxymethyl) phosphonium phosphate.

The condensate is preferably a condensate of tris(hydroxyorgano)phosphine with a nitrogen containing compound.

The nitrogen containing compound is preferably urea. Alternatively the nitrogen containing compound is selected from a C<sub>1-20</sub> alkylamine, dicyandiamide, thiourea and guanidine

5 Alternatively the invention provides the use as an uncoupling agent a water-soluble biocide that comprises a compound selected from the following:

- 10       • quaternary ammonium compounds, e.g. dodecyl trimethyl ammonium chloride, cetyltrimethylammonium bromide, benzalkonium chloride, didecyldimethylammonium chloride and alkyldimethylbenzylammonium chloride;
- polymeric quaternary ammonium compounds, e.g. polyoxyethylene (dimethylimino) ethylene dichloride;
- 15       • polymeric biguanide hydrochlorides, e.g. polyhexamethylenebiguanide hydrochloride, dodecylguanidine hydrochloride;
- tris(hydroxymethyl)nitromethane;
- 4,4-dimethylozazolidine;
- 20       • phenoxypropanol;
- phenoxyethanol;
- glyoxal;
- acrolein;
- aldehydes, e.g. formaldehyde, glutaraldehyde;
- 25       • triazines, e.g. 1,3,5-tris(2-hydroxyethyl)-1,3,5-hexahydrotriazine;

- quaternary phosphonium compounds, e.g. tributyltetradecylphosphonium chloride and tetradecyl tributyl phosphonium chloride;
- 2-bromo-4-hydroxyacetophenone;
- 5 • carbamates, e.g. sodium N-dimethyldithiocarbamate, disodium ethylene bisdithiocarbamate;
- tertbutylazine;
- tetrachloro-2,4,6-cyano-3-benzonitrile;
- thiazole and isothiazole derivatives such as 2-methyl-4-  
10 isothiazolin-3-one, 5-chloro-2-methyl-4-isothiazolin-3-one, 5-chloro-2-methyl-3(2H)-isothiazolone and 1,2-benzisothiazolin-3-one;
- compounds with activated halogen groups such as 2-bromo-2-  
15 nitro-propan-1,3-diol and 2,2-dibromo-3-nitrilopropionamide.
- bis chloromethyl sulphone, and
- methylene bis thiocyanate.

20 The water-soluble biocide for use as an uncoupling agent may be formulated with one or more of the following chemicals conventionally used in wastewater treatment:

- a surfactant;
- an antifoam;
- 25 a scale inhibitor;
- a corrosion inhibitor;
- a biocide;

a flocculant;  
a dewatering aid, and  
a dispersant.

- 5 Preferably, the aqueous system will be a wastewater treatment plant that is used for the treatment of industrial or municipal effluent. Such a plant typically takes in wastewater from industrial processes (e.g. paper production, food processing, chemical industry) and/or domestic and institutional habitations and the like and, by using micro-organisms in  
10 aerobic, anoxic (e.g. denitrification) and/or anaerobic processes, to consume organic contaminants and render the water fit for re-use or discharge into the environment.

- The present invention also provides the use as an uncoupling agent of  
15 conventional, water-soluble, water treatment biocides as defined above.

The present invention further provides an uncoupling agent comprising one or more conventional water-soluble, water treatment biocide(s) as defined above.

- 20 The present invention further provides a method for controlling the growth of bacterial biomass in an aqueous system comprising adding to, or contacting with, the aqueous system an effective amount of an uncoupling agent which is a water-soluble biocide as defined above.

- 25 In a preferred embodiment the present invention provides a method for controlling the growth of bacterial biomass in an aqueous system, which method comprises contacting an effective amount of an uncoupling agent which is a water-soluble biocide as defined above directly with the  
30 bacterial biomass.

The effective amount of the water-soluble biocide added to the aqueous system is up to 5000mg/l, for example up to 3000mg/l, such as up to 1000mg/l. Preferably, the effective amount of the water-soluble biocide added to the aqueous system is from 0.005 mg/l to 500 mg/l, for example  
5 from 0.01mg/l to 300mg/l, such as from 0.05mg/l to 100mg/l. More preferably, the effective amount of water-soluble biocide is from 0.1 to 10 mg/l, for example from 0.5mg/l to 7.5mg/l, such as from 1 to 5mg/l. Alternatively, the effective amount of said water-soluble biocide may be from 0.1 to 10,000 milligrams per gram of sludge solids in the aqueous  
10 system. Preferably, from 0.5 to 1000mg/g, for example, from 1 to 500mg/g, such as from 5 to 100mg/g.

Contacting the bacterial biomass with the water-soluble biocide directly has been found to lead to improved efficiency of the uncoupling agent in  
15 controlling bacterial biomass. The direct contact of the water-soluble biocide with bacterial biomass is termed "flash dosing" or "flash mixing". It has been found that if the uncoupling agent is simply added directly to a bioreactor containing sludge then the efficacy of the agent is substantially reduced as the uncoupling agent is able to interact with other  
20 matter in the bioreactor and the action of the water-soluble biocide is substantially reduced.

The invention will now be described merely by way of example with reference to the following examples and accompanying figures, of which:  
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Figure 1 is a diagram illustrating the process of flash mixing;

Figure 2 is a diagram of a semi-continuous chemostat reactor system used in the present invention;



Figure 3 is a diagram of an Oxitop® BOD system used to obtain oxygen consumption data, and

Figure 4 is a graph showing percentage uncoupling of four uncoupling  
5 agents.

Referring to figure 1 a typical bioreactor B is shown with a recirculating line R into which the water-soluble biocide is injected. The bioreactor contains sludge, which is essentially a mass of typical wastewater  
10 treatment microorganisms such as bacteria, protozoa, worms and fungi. The bioreactor B has an inlet I for wastewater and an outlet O for treated water. The sludge is extracted from the bioreactor into the recirculation line R by means of a pump P. Typically, a peristaltic pump is used. The candidate water-soluble biocide is introduced to the sludge in the  
15 recirculating line via a needle connected to a syringe pump N. It will be appreciated that in a commercial scale bioreactor the water-soluble biocide will be introduced via a tee-junction or an injection quill and a progressive cavity pump may replace the peristaltic pump. This enables direct contact of the sludge with the water-soluble biocide. The water-  
20 soluble biocide may be introduced into the recirculation line R at a location behind or ahead of the pump P.

However, it will be appreciated that the implementation of the invention is not restricted to using the method of "flash mixing" and other dosing  
25 methods may be used if so desired.

### Example 1 - Semi-Continuous Pilot Study with Flash Mixing

#### 1. Equipment

30

A series of semi-continuous chemostat reactors was constructed to

simulate wastewater treatment processes. The arrangement of a semi-continuous chemostat reactor is shown in figure 2.

The central part of the equipment is the biological chemostat reactor 1.

- 5 The reactor vessel 2 is made of glass, with an internal volume of 10 litres and is fitted with pH 3 and dissolved oxygen 4 sensors and an air sparge 5. This contains the sludge, which is, essentially, a mass of typical wastewater treatment microorganisms. The reactor operates at an ambient temperature (approximately 20°C).

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Simulated wastewater 13 is contained in a separate vessel 6 and maintained at 4°C to prevent microbial spoilage. It is transferred into the reactor via a supply line 7 and a peristaltic pump 8.

- 15 A recirculating line 9 is provided on the reactor comprising a length of flexible silicone tubing and a peristaltic pump 10. The peristaltic pump extracts the sludge from the reactor into the recirculation line. The candidate water-soluble biocide is introduced into this line 9 through a needle 11, which is fed by a syringe pump 12. Using this system it is possible to achieve "flash mixing" which ensures rapid contact of the water-soluble biocide with the sludge microorganisms. The internal diameter of the recirculating line was 8 mm and the flow velocity was 0.5 metres per second, which gave a contact time, between candidate water-soluble biocide and sludge of 3 seconds before it re-entered the reactor.
- 20

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Treated effluent 17 is removed from the reactor into a collecting vessel 16 via an outlet line 15.

## 2. Procedure

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The reactor was charged with sludge obtained from the Municipal wastewater treatment works at Courly - Lyon, in France. This sludge provided the "seed" to start the process off. The reactor was then filled, to the 5 litre mark, with simulated wastewater, adjusted to approximately pH 7.5, and aeration was commenced. The reactor was continuously fed, at a rate of 800 ml per day, with simulated wastewater made to the recipe shown in Table B.

Each weekday (i.e. 5 times per week), 13.3% of the bioreactor contents were withdrawn to maintain the equilibrium. This ensured a sludge age in the region of 7.5 days.

Also, each weekday, after the adjustments described in the preceding paragraph, candidate water-soluble biocide (as a dilute solution) was injected into the system via the flash mixing recirculating line. The dilute solution was prepared by dissolving 10 grams of sodium hydrogen carbonate in approximately 900 ml of deionised water then adding 5 grams of candidate water-soluble biocide and mixing well. The mixture was then made up to 1 litre with deionised water. The appropriate volume of this dilute solution was injected to achieve the required dose level.

The experiment was conducted for 52 days before decommissioning the system.

### 3. Test Concentrations

Compound X was evaluated in the test. This compound is a 75% aqueous solution of tetrakis(hydroxymethyl)phosphonium sulphate (THPS). Two concentrations were evaluated: 2 and 4 milligrams per litre per day.

#### 4. Results

Table A

Treatment	Specific Activity (g COD/g MLSS.d)	Growth Yield (gMLSS/gCOD)	Growth Yield Reduction (%)
No Treatment (Control)	0.477	0.272	0
Compound X, 2 mg/l/day	0.582	0.202	27.7
Compound X, 4 mg/l/day	0.772	0.167	37.6

\* Specific Activity is the rate of COD removal (grams per day) divided

5 by the quantity of sludge solids (grams) present in the reactor.

Also, the following observations were made during the pilot study:

- 10 • The control (untreated) study showed a proliferation of filamentous bacteria in the sludge whereas these were substantially absent in sludge from Compound X treated systems.
- 15 • There was a significantly higher concentration of protozoans and other higher organisms in the treated sludge relative to the untreated.
- Compound X treatment did not impair sludge flocculation.

#### 5. Comments

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This study confirmed that Compound X flash treatment, at low dose levels, was capable of significantly reducing sludge growth whilst actually increasing the specific activity of the sludge.

Also, sludge quality was significantly improved resulting in a sludge which would flocculate more easily (in case of filamentous bacteria proliferation), thus providing operational benefits.

## 5 Simulated Wastewater Composition

Table B

Component	Concentration (mg/l)
Acetic Acid	3400
Sucrose	600
Yeast Extract	1200
Casein Peptone	600
Potassium Sulphate	180
Magnesium Sulphate, heptahydrate	180
Ferrous Sulphate, heptahydrate	30
Calcium Chloride, anhydrous	20
Disodium Hydrogen	280
Sodium Hydrogen Carbonate	quantity to adjust to pH 4.3

## Example 2 - Semi-Continuous Pilot Study with Pre-Dilution

### 10 1. Equipment

The equipment used in this test sequence was identical to that used for example 1 except that the reactor was not fitted with a recirculating line for flash mixing.

15

### 2. Procedure

The procedure was identical to that used for example 1 except that the method of dosing Compound X was different. In this case, 5 grams of Compound X was mixed into a solution containing 5 grams of sodium hydrogen carbonate in 1 litre of deionised water. 4.0 ml of this solution was added to 500 ml of clarified wastewater which had been

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extracted from the bioreactor. The treated clarified wastewater was then returned to the bioreactor and normal operation of the bioreactor was resumed. The final concentration of Compound X in the bioreactor therefore equates to 4 mg per litre. This procedure was  
5 conducted daily, 5 times per week.

The experiment was conducted in parallel with example 1, i.e. for 52 days before decommissioning the system.

### 10 3. Test Concentration

A Compound X concentration of 4 milligrams per litre per day was used.

### 15 4. Results

Table C

Treatment	Specific Activity (g COD/gMLSS.d)	Growth Yield (gMLSS/gCOD)	Growth Yield Reduction (%)
No Treatment (Control)	0.477	0.272	0
Compound X, 4 mg/l/day	0.636	0.201	26.4

\* Specific Activity is the rate of COD removal (grams per day) divided by the quantity of dry sludge solids (grams) present in the reactor.

### 20 5. Comments

The reduction in growth yield in this test was only 26.4% as compared to the 37.6% reduction (see example 1), at the same dose rate, when flash mixing was used. This demonstrates the benefit of flash mixing.

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### Example 3 - Uncoupling by Other Water Treatment Biocides

#### 1. Summary

A laboratory screening test was developed to determine the impact of chemical compounds on respiration parameters through an oxygen consumption pattern analysis using a model based on published work by Monod and Pirt<sup>1</sup>. Growth uncoupling is characterised by an increase in oxygen consumption during bacterial growth in a synthetic growth medium.

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#### 2. Principle

Batch growth kinetic modelling and the interpretation of the oxygen consumption kinetics were implemented by mathematical modelling.

15 Growth kinetics is described, according to Monod and Pirt's model, as follows:

$$\frac{d [ X ]}{dt} = a \cdot \frac{d [ S ]}{dt} - b \cdot [ X ]$$

$$\frac{d [ S ]}{dt} = k \cdot [ X ] \cdot \frac{[ S ]}{K_s + [ S ]}$$

$$\frac{d [ O_2 ]}{dt} = a \cdot \frac{d [ S ]}{dt} + b \cdot [ X ]$$

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1. Monod J. 1950, Ann Inst Pasteur, "La Technique de Culture Continue, Theorie et Applications", 79:390-410. Monod J. 1942, "Recherches sur la Croissance des Cultures Bactériennes". Paris Herman p. 210. Pirt S.J. (1965) "The Maintenance Energy of Bacteria in Growing Cultures". Proceedings of the Royal Society of London B 163, 224-231. "Principles of Water Quality Management". Ed: W.W. Eckenfelder, Jr. CBI Publishing Company, Inc USA. 1980 : Chapter 9 - Biological Waste Treatment pp 249-275.

- [X] : Concentration in biomass amount subject to endogenous metabolism, g MLSS/L
- [S] : Carbon substrate concentration, g/L
- d[X]/dt : Biomass growth rate, g dry biomass/L.d
- 5 d[S]/dt : Carbon substrate consumption rate, g /L.d
- a : Intrinsic substrate-biomass growth yield, g X/g substrate consumed
- b : Biomass maintenance coefficient, g dry biomass/g dry biomass. d
- 10 d[O<sub>2</sub>] /dt : Oxygen consumption rate, g O<sub>2</sub>/L.d
- a' : Specific oxygen consumption coefficient, g O<sub>2</sub>/g organic substrate consumed.
- b' : Endogenous respiration coefficient, g O<sub>2</sub>/g X
- k : Maximum specific activity, g S/ g dry biomass.d
- 15 K<sub>s</sub> : Apparent substrate affinity coefficient or half maximum specific activity substrate concentration, mg/l

Relationship between a and a' :  $a' + (b'/b) * a = 1$

Relationship between b and b' :  $b' = 1.25 * b$

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For the evaluation of proposed water-soluble biocides, the oxygen consumption kinetics are measured and, a process of computer iteration optimises the equation coefficients optimised to fit the oxygen consumption curve.

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From the optimised coefficients, the degree of bacterial uncoupling can be calculated.

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### 3. Equipment

An Oxitop® BOD System, supplied by Wissenschaftlich-Technische  
Werkstätten GmbH, Dr.-Karl-Slevost-Strasse 1, D-82362 Weilheim,  
5 Germany, was used for the screening tests (see figure 3). The complete  
system (see figure 3) comprised:

- 500 ml glass bottles 20 containing seeded culture medium 21;
- Each bottle was capped with a screw-on gas pressure meter 22  
capable of recording up to 360 pressure data points. Sodium  
10 hydroxide pellets 23 were incorporated into the system to absorb  
carbon dioxide produced during bacterial respiration.
- The culture medium 21 was stirred using a magnetic stirrer 25 and  
a magnetic flea 24.
- The bottles were housed in a temperature controlled container  
15 (20°C)
- Data acquisition was by infra-red link with a hand-held control  
unit.
- Data was finally transferred from the hand-held control unit to an  
Excel® spreadsheet file for the calculations to be performed.

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### 4. Test Biocides

Three conventional water treatment biocides were evaluated as bacterial  
water-soluble biocides. Each represented a major class of biocide type  
25 used conventionally in the water treatment industry. In addition, 2,4-  
dinitrophenol, a conventionally known bacterial water-soluble biocide was  
included in the test sequence for comparison. Details, as follows:

Table D

Test Substance	Code	Class of biocides
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		represented
2,4 Dinitrophenol	A	-
Glutaraldehyde (15% active ingredient)	B	Aldehydes
Dodecyltrimethylammonium chloride (laboratory chemical)	C	Quaternary ammonium salts
Blend of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (1.5% active ingredient)	D	Isothiazolones

### 5. Test Concentrations

Based on the liquid sludge, 2,4-dinitrophenol was dosed at 20 mg/l, this being  
 5 the known level at which significant uncoupling would result. The test biocides were each evaluated at 1, 2.5, 5, 10 and 25 mg/l.

### 6. Procedure

10 Growth medium:

Glucose: 500 mg/l  
 Yeast extract Difco: 50 mg/l  
 Mineral nutrients: as described in Standard Method ISO 9888  
 15 (Determination of ultimate aerobic biodegradability in aquatic environment)

Medium seeding:

20 50 mg/l homogenized and washed sample from municipal activated sludge treatment unit.

**Incubation:**

5        7 days at 20 °C in BOD-meter bottles (OxiTop)

The oxygen consumption was automatically determined and recorded all through the growth and decay phases (a total of 360 data points for each test substance).

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Data was interpreted using a simple growth mathematical model as described above.

The determination of the model's coefficients are made through  
15 adjustment and using the statistical test  $\chi^2$  to fit the model to the experimental data.

$$\chi^2 = \sum \frac{[OC_{\text{observed}} - OC_{\text{theoretical}}]^2}{OC_{\text{theoretical}}}$$

20    where  $OC$  is the oxygen consumption.

**7. Results**

Figure 4 summarises the results and shows that all three test biocides produced  
25 significant and useful levels of uncoupling. Two of them (biocides C and D) produced greater levels of uncoupling at significantly lower dose levels than the conventional water-soluble biocide, 2,4-dinitrophenol. However, it should be borne in mind that these were screening tests and conditions were not optimised for the individual biocides and so it is possible that biocide B might have  
30 produced greater uncoupling under more optimum conditions.

## 8. Comments

The results clearly show that representatives of three major water  
5 treatment biocide classes will effect significant bacterial uncoupling at  
low treatment levels.

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## CLAIMS

1. The use of a water-soluble biocide as an uncoupling agent at an effective amount to control bacterial biomass in an aqueous system.

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2. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 1 in which the effective amount of the uncoupling agent is up to 5000 mg/l.

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3. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 2 in which the effective amount of the uncoupling agent is up to 3000 mg/l.

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4. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 3 in which the effective amount of the uncoupling agent is up to 1000 mg/l.

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5. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 4 in which the effective amount of the uncoupling agent is from 0.005mg/l to 500mg/l.

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6. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 5 in which the effective amount of the uncoupling agent is from 0.01mg/l to 300mg/l.

7. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 6 in which the effective amount of the uncoupling agent is from 0.05mg/l to 100mg/l.

8. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 3 in which the effective amount is from 0.1mg/l to 10mg/l.

5 9. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 8 in which the effective amount of the uncoupling agent is from 0.5mg/l to 7.5mg/l.

10 10. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 9 in which the effective amount of the uncoupling agent is from 1mg/l to 5mg/l.

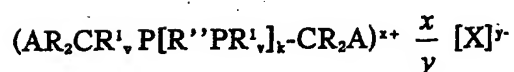
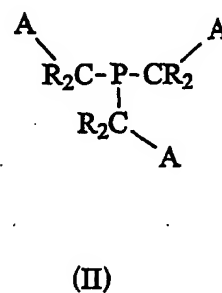
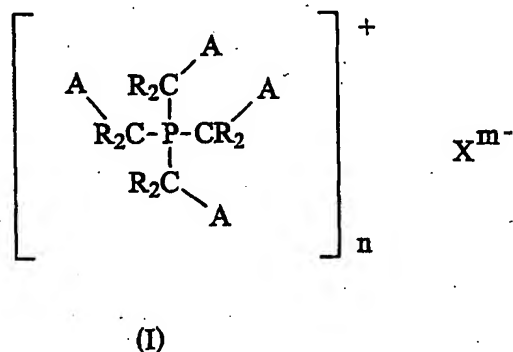
11. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 1 in which the effective amount of the uncoupling agent  
15 is from 0.1mg to 10000mg per gram of sludge solids in the aqueous system.

12. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 11 in which the effective amount of the uncoupling agent  
20 is from 0.5mg to 1000mg per gram of sludge solids in the aqueous system.

13. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 12 in which the effective amount of the uncoupling agent  
25 is from 1mg to 500mg per gram of sludge solids in the aqueous system

14. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 13 in which the effective amount of the uncoupling agent is from 5mg to 100mg per gram of sludge solids in the aqueous system

15. The use of a water-soluble biocide as an uncoupling agent as claimed in any one of the preceding claims in which the water-soluble biocide comprises an alkyl substituted phosphonium compound of formula (I) or an alkyl substituted phosphine of formula an alkyl-substituted phosphine of formula (II) and a condensate of formula (III):



(III)

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wherein:

X is an anion;

n is the valency of X represented by m;

each A can be the same or different and is selected from OH, OR, SO<sub>3</sub>R, PO<sub>3</sub>R<sub>2</sub>, COOH, COOR, SO<sub>3</sub>H, PO<sub>3</sub>H<sub>2</sub>, CH<sub>2</sub>COOH, substituted alkyl, aryl and substituted amino groups;

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each R, and each R in each A group, is independently selected from hydrogen, a C<sub>1</sub> to C<sub>20</sub> alkyl, aryl, substituted alkyl or aryl, carboxy or carboxy ester; wherein each CR<sub>2</sub> group may be the same or different, and

- 5 R'' is a divalent hydrocarbon radical having from 2-20 carbon atoms and is optionally substituted with one or more substituents selected from the group consisting of halogen, hydroxy, carboxy, amino, alkylamino, or PR<sup>1</sup><sub>m</sub>CH<sub>2</sub>OH groups or interrupted by one or more ether or carbonyl linkages;

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each R<sup>1</sup> is independently a monovalent hydrocarbon radical having from 1 to 25 carbon atoms and optionally substituted with one or more substituents selected from the group consisting of halogen, hydroxy, carboxy, amino, alkylamino, or PR<sup>1</sup><sub>m</sub>CH<sub>2</sub>OH groups or interrupted by one or more ether or carbonyl linkages, and

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in formula (III) each v is 1 or 2, k is from 0 to 10 (e.g. from 1 to 10), x is the number of groups in the molecule having v=2 and X is a compatible anion of valency y such that the compound is water-soluble.

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16. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 15 wherein X is selected from the group consisting of chloride, sulphate, phosphate, acetate and bromide.

25

17. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 15 or claim 16, wherein the alkyl-substituted phosphonium compound is tetrakis (hydroxymethyl) phosphonium sulphate.

30

18. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 15 or claim 16 wherein the alkyl-substituted



phosphonium compound is selected from a group consisting of tetrakis (hydroxymethyl) phosphonium chloride, tetrakis (hydroxymethyl) phosphonium bromide, tetrakis (hydroxymethyl) phosphonium acetate and tetrakis (hydroxymethyl) phosphonium phosphate.

5

19. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 15 in which the condensate is a condensate of tris(hydroxyorgano)phosphine with a nitrogen containing compound.

10 20. The use of a water-soluble biocide as an uncoupling agent as claimed in claim 19 in which the nitrogen containing compound is selected from the group consisting of a C<sub>1-20</sub> alkylamine, dicyandiamide, thiourea and guanidine.

15 21. The use of a water-soluble biocide as an uncoupling agent as claimed in any one of claims 1 to 14 wherein the uncoupling agent comprises a compound selected from the group consisting of quaternary ammonium compounds; polymeric quaternary ammonium compounds; polymeric biguanide hydrochlorides; tris(hydroxymethyl)nitromethane;  
20 4,4-dimethylazolidine; phenoxypropanol; phenoxyethanol; glyoxal; acrolein; aldehydes; triazines; quaternary phosphonium compounds; 2-bromo-4-hydroxyacetophenone; carbamates; tertbutylazine; tetrachloro-2,4,6-cyano-3-benzonitrile; thiazole and isothiazole derivatives; compounds with activated halogen groups; bis chloromethyl  
25 sulphone, and methylene bis thiocyanate.

22. The use of a water-soluble biocide as an uncoupling agent as claimed in any one of the preceding claims in which the water-soluble biocide is formulated with one or more of a surfactant; an antifoam; a  
30 scale inhibitor; a corrosion inhibitor; a biocide, a flocculant, a dewatering aid and a dispersant.

23. The use of a water-soluble biocide as an uncoupling agent as claimed in any one of the preceding claims wherein the aqueous system is a wastewater treatment plant used for the treatment of industrial or municipal effluent.

5

24. An uncoupling agent comprising one or more conventional, water-soluble, water treatment biocide(s) as claimed in any one of claims 15 to 21.

10 25. A method for controlling the growth of bacterial biomass in an aqueous system comprising adding to, or contacting with, the aqueous system an effective amount of an uncoupling agent which is a water-soluble biocide as defined in any one of claims 15 to 21.

15 26. A method as claimed in claim 25 in which the method comprises contacting an effective amount of a water-soluble biocide directly with the bacterial biomass.

20 27. A method as claimed in claim 25 or claim 26 in which the effective amount of the water-soluble biocide added to the aqueous system is up to 5000 mg/l.

28. A method as claimed in claim 27 in which the effective amount of the water-soluble biocide added to the aqueous system is up to 3000 mg/l.

25

29. A method as claimed in claim 28 in which the effective amount of the water-soluble biocide added to the aqueous system is up to 1000 mg/l.

30 30. A method as claimed in claim 29 in which the effective amount of the water-soluble biocide added to the aqueous system is from 0.005 mg/l to 500 mg/l.

31. A method as claimed in claim 30 in which the effective amount of the water-soluble biocide added to the aqueous system is from 0.01 mg/l to 300 mg/l.
- 5 32. A method as claimed in claim 31 in which the effective amount of the water-soluble biocide added to the aqueous system is from 0.05 mg/l to 100 mg/l.
33. A method as claimed in claim 32 in which the effective amount of  
10 the water-soluble biocide is from 0.1 mg/l to 10mg/l.
34. A method as claimed in claim 33 in which the effective amount of the water-soluble biocide added to the aqueous system is from 0.5 mg/l to 7.5mg/l.
- 15 35. A method as claimed in claim 34 in which the effective amount of the water-soluble biocide added to the aqueous system is from 1mg/l to 5mg/l.
- 20 36. A method as claimed in claim 25 or claim 26 in which the effective amount of the water-soluble biocide added to the aqueous system is from 0.1 mg to 10000mg per gram of sludge solids in the aqueous system.
37. A method as claimed in claim 36 in which the effective amount of  
25 the water-soluble biocide added to the aqueous system is from 0.5 mg to 1000mg per gram of sludge solids in the aqueous system.
38. A method as claimed in claim 37 in which the effective amount of the water-soluble biocide added to the aqueous system is from 1 mg to  
30 500mg per gram of sludge solids in the aqueous system.

39. A method as claimed in claim 38 in which the effective amount of the water-soluble biocide added to the aqueous system is from 5mg to 100mg per gram of sludge solids in the aqueous system.

5 40. The use of a water-soluble biocide as an uncoupling agent substantially as described herein with reference to the accompanying examples.

10 41. An uncoupling agent comprising a conventional, water-soluble, water treatment biocide substantially as described herein with reference to the accompanying examples

15 42. A method of controlling the growth of a bacterial biomass in aqueous systems substantially as described herein with reference to the accompanying examples.

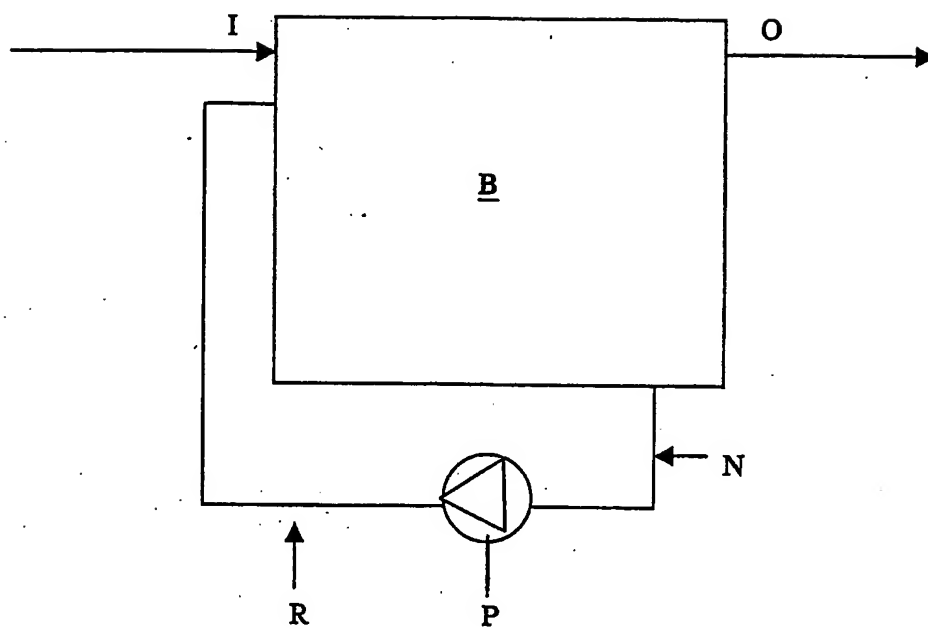


Figure 1

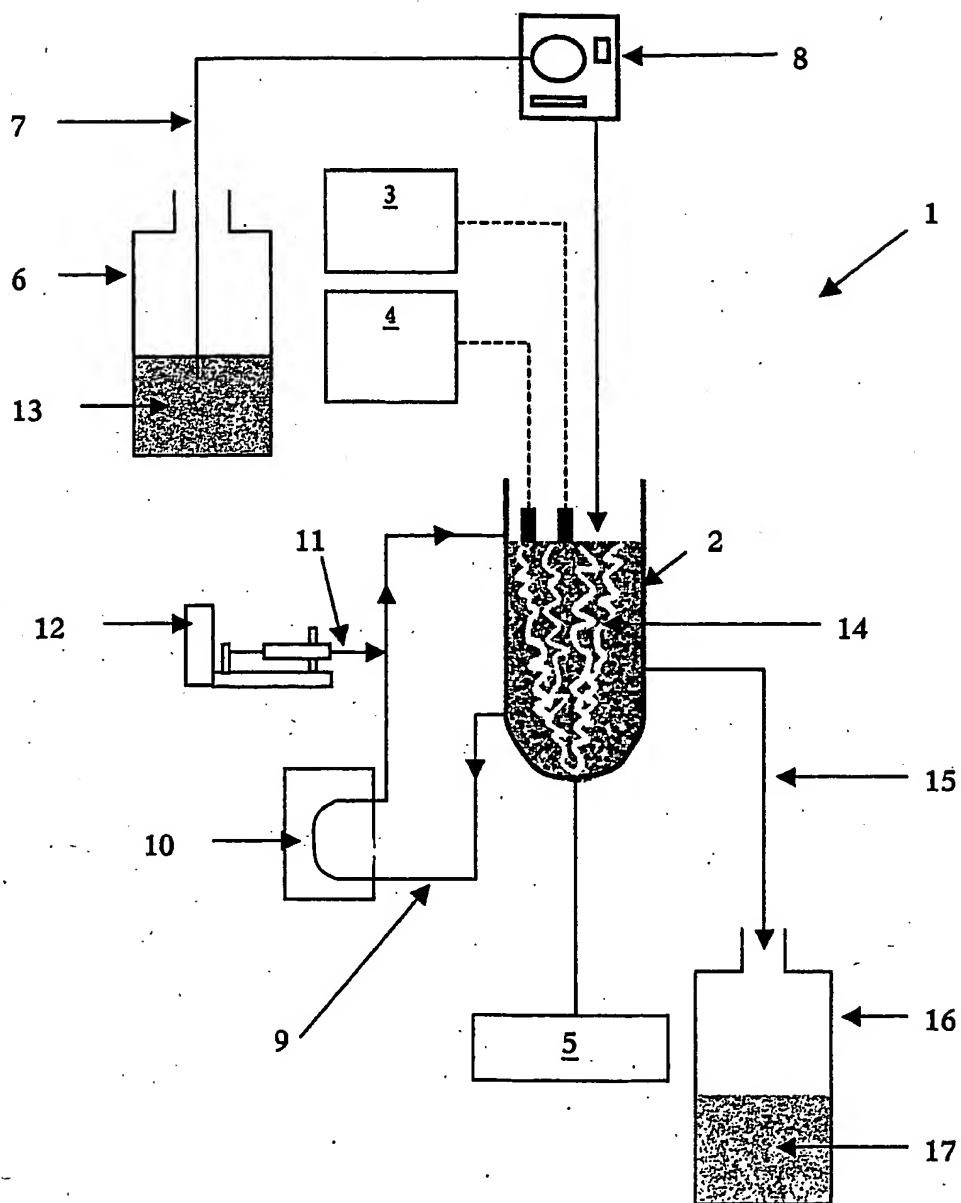
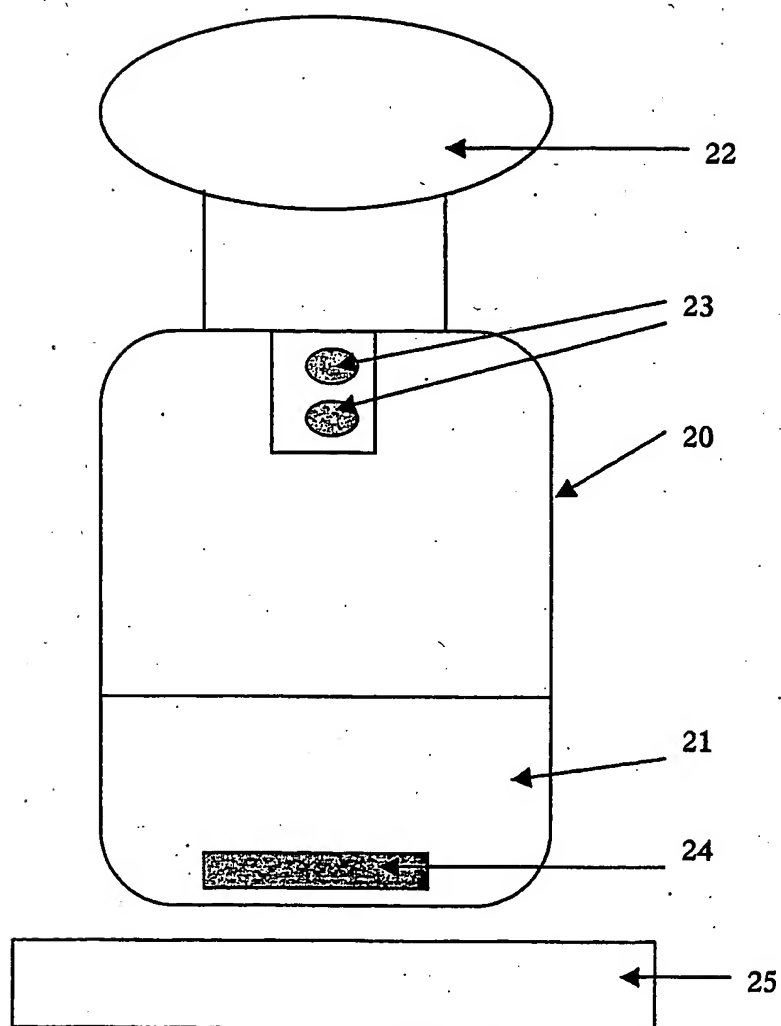


Figure 2

Figure 3



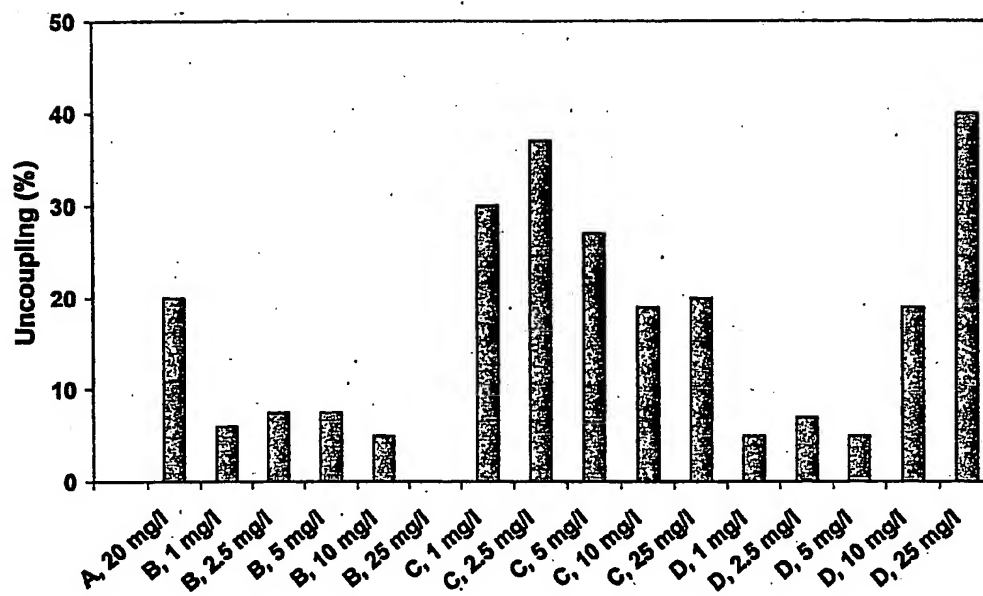


Figure 4